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Aurélie Charazac, Célia Decondé Le Butor, Mamadou Gueye, Jérôme Gilleron, Kévin Giuletti, et al.. Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.. Signalife, May 2017, Nice, France. pp.1. hal-01548770

**HAL Id: hal-01548770**

**<https://hal.science/hal-01548770>**

Submitted on 28 Jun 2017

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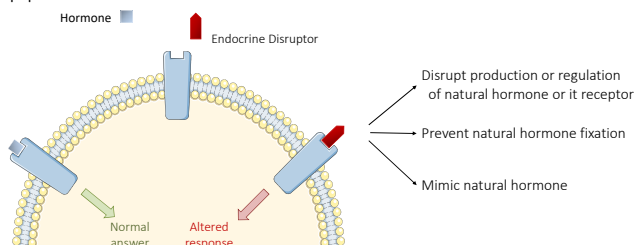
# Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.

Charazac Aurélie<sup>1</sup>, Decondé le Butor Célia<sup>1</sup>, Gueye Mamadou<sup>1</sup>, Gilleron Jérôme<sup>1</sup>, Giuletti Kevin<sup>3</sup>, Gesson Maeva<sup>1</sup>, Fénichel Patrick<sup>2</sup>, Descombes Xavier<sup>3</sup>, Bost Frédéric<sup>1</sup>, Clavel Stéphan<sup>1</sup> & Chevalier Nicolas<sup>2</sup>.

1) Institut National de la Santé et de la Recherche Médicale (INSERM) UMR U1065/UNS, Centre Méditerranéen de Médecine Moléculaire (C3M), 06202 Nice cedex 3, France  
2) Centre Hospitalier Universitaire de Nice, Hôpital de l'Archet 2, Service d'Endocrinologie, Diabétologie et Médecine de la Reproduction, 151 route de Saint-Antoine de Ginestière, CS 23079, 06202, Nice Cedex 3, France  
3) INRIA CRI-SAM, 2004 route des Lucioles, 06902, Sophia Antipolis Cedex, France

## Endocrine disruptors

According to the World Health Organization, an endocrine disruptor may be described as an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny, or (sub) populations.



They are found in many everyday products like food packaging, food preservatives or additives, pesticides residues, everyday plastic objects, etc.

Widely distributed throughout the environment and bioaccumulable in living organisms, Persistent Organic Pollutants are a specific class of EDC that accumulate in fat deposit. Some of them have been recognized as causing adverse effects on wildlife and also human's health such as diabetes and cancer...

## Working hypothesis

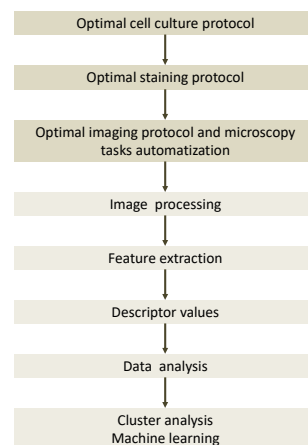
Cancer cells undergo of genetic modifications that lead to a phenotype characterized by a high metabolic flexibility allowing them to grow in various cellular environments and favoring their proliferative and invasive capacities as well as death resistance. Mitochondria are complex organelles possessing a central role in metabolism, apoptosis, signaling, etc.

### AIM

Develop screening procedures to analyze the effects of various endocrine disruptors at different concentrations and in combination on:

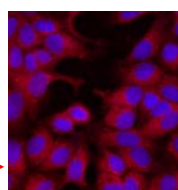
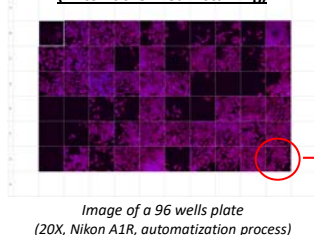
- **Mitochondrial morpho-function**  
(High throughput screening + image analysis and classification)
- **Metabolism**  
(XF analyzer, SeaHorse)
- **Migration/Invasion/Proliferation**  
(Videomicroscopy, IncuCyte)
- **Signaling pathways**  
(Luminex)

## Workflow for microscopy based screening

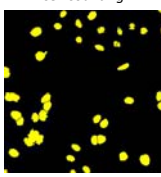


## Microscopy based analysis of mitochondria functional parameters

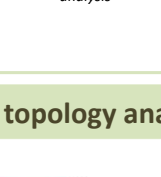
### Mitochondrial membrane potential (MitoTracker Red™ staining)



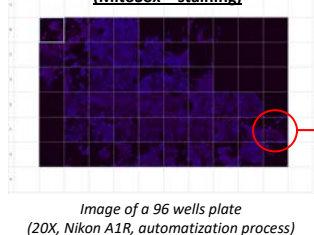
1) Nuclear masking for cell counting



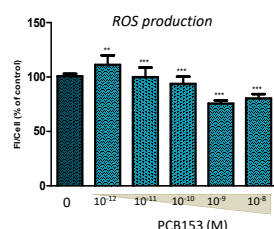
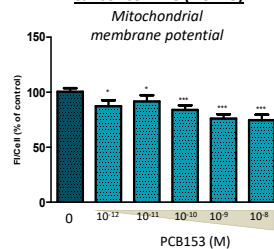
2) Dye masking for cell fluorescence intensity analysis



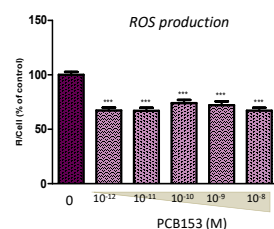
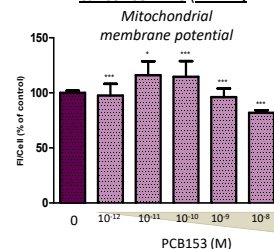
### Superoxide anion production (MitoSox™ staining)



### Androgen insensitive prostate cancer cell line (DU145)

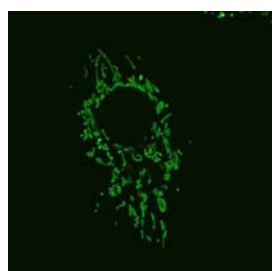


### Androgen sensitive prostate cancer cell line (22RV1)



Example of results obtained using this quantitative image based analysis on DU145 and 22RV1 prostate cancer cell lines after treatment with a range doses of PCB153, an ED non inflammable electric isolant.

## Mitochondrial topology analysis and classification



Original picture  
(60X, Nikon A1R)

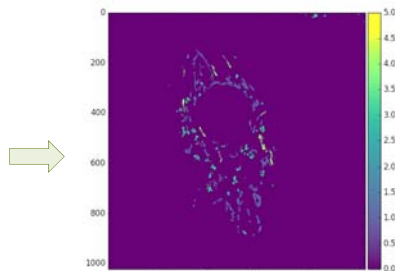
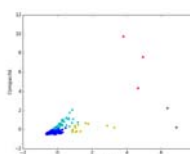
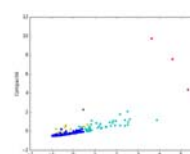


Image with developed method  
(Python program)

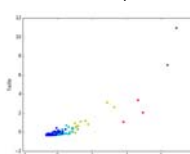
Classification



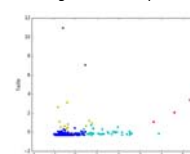
Form vs compactness



Elongation vs compactness



Form vs size



Elongation vs size



Clusterization of  
mitochondrial shape

The developed method using Python will classified all image's region according to the highest gain leading to no loose of information or noisy image. After detection, this method will automatically partitioning regions using K-means methods leading to the clusters classifications thanks to following criterias : form, compactness, size, elongation, fragmentation.

When combined, morphological and functional parameters allow us to discriminate subtle perturbations of the mitochondrial structure-function induced by endocrine disruptors in prostate cancer cells. We are confident that this multiparameter analysis strategy could represent a new perspective in identification and characterization of endocrine disruptors based on their effects on cell metabolism in order to estimate their potential risk on human health. In the future, this can lead to a high-throughput screening method useful to industrial and regulatory agencies.

Don't hesitate to contact us for  
collaboration!  
aurelie.charazac@unice.fr